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BIOLOGICAL BULLETIN

SELECTIVE FERTILIZATION IN POLLEN MIXTURES.

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Equality in fertilizing power of gametes dissimilar in the hereditary factors they carry is a corollary of Mendelism. It has been thought that certain exceptions to this general rule may exist. Differential fecundating ability has been suggested from time to time as a possible interpretation of otherwise unaccountable results. The earlier uses of this hypothesis of inequality in sperm efficiency, such as Castle's (1903) theory of sex determination, Cuenot's (1908) conception of the reason for the non-appearance of homozygous yellow mice, have since been found to be unnecessary. The interpretation of linkage phenomena according to the reduplication hypothesis, while essentially different in application, holds somewhat the same implication as selective fertilization, that is, differential operative power of cells of unlike germinal construction.

As the yellow mouse problem was finally resolved to a selective elimination of zygotes so many puzzles have had considerable light thrown on them by a more complete understanding of the factor relations. Many instances of the non-appearance of certain types are known to be the result of the action of lethal factors. Good illustrations of this are found in *Drosophila* (Muller, 1918). Other cases of elimination immediately after fertilization or early in development are known in maize.

Sometimes abortion of a part of the gametes before fertilization takes place and it is assumed that the elimination is selective. Belling (1914) has shown that in *Stizolobium* 50 per cent. of the pollen grains and ovules regularly abort in certain types. The aberrant results from the *Oenotheras* are now generally considered largely, if not wholly, to be due to differential destruction of both

gametes and zygotes. Davis (1915-'17) has called attention to the great amount of pollen and ovule abortion in these plants and to the low germination of the seeds which are produced and to the results which are obtained when a more complete germination is secured.

It is not always easy to distinguish between differential destruction of zygotes immediately after fertilization and selective fertilization. It is still more difficult in the case of abortion of germ cells. In fact it is only a matter of degree between selective elimination of gametes and selective fertilizing ability. From germ cells which are unable to complete development on account of the particular inheritances they carry, to gametes which appear normal but are unable to function perfectly under any circumstances, it is only a step. However, no instance of the latter condition is positively known and the cases of gametic abortion clearly due to the factors which the gametophytes carry are rare.

Although results still remain to be cleared up which indicate a selective action of some kind (Kempton, 1919) the general conclusion holds, which is, that the pollen, although gametophytic itself, has the function of the sporophyte which produces it. This is supported by the well-known cases where pollen color and shape are all of the type of the maternal parent irrespective of the factors for pollen characters which they carry. Many instances are known where gametes, containing lethal factors which stop development immediately after fertilization, are able to function fully as well as others permitting normal growth. East and Park (1917) have shown that compatibility in *Nicotiana* is alike for all the gametes of any one individual. Different pollen grains may carry factors which will determine sterility or fertility of the future zygotes but all function alike in fertilization according to the sporophyte from which they come. Following up these results East (1919) shows that in the frequency distributions of pollen tube lengths there is no significant difference between pollen from plants greatly unlike in the degree of heterozygosity. In other words gametes carrying markedly diverse germinal complexes are no more variable than those all of like constitution in ability to send pollen tubes down the styles

and to accomplish fertilization. East considers that chance segregation in the germ cells and random mating of these germ cells is a fundamental genetic hypothesis applicable to plants and animals alike. With this conclusion the writer is in accord, with the reservation that the evidence from gametic abortion should not be put aside as belonging to a different category.

The data to be presented here bear upon another phase of the problem. The above conclusion, it should be clearly kept in mind, applies only to gametes produced by one or more individuals of the same type. That is, the gametes may be unlike in the factors they carry, but if they come from the same or similar individuals they are potentially equal in ability to fertilize. But what is the result when germ cells from two individuals of different type are presented at the same time in excess so that not all can fulfill their function? Will fertilization take place at random or not? This is the problem to be considered here. In the one case doses of different kinds of medicine come in the same capsules. In the other the capsules as well as their contents may differ. Anyone accustomed to swallowing a particular kind of capsule made of a familiar substance and all of the same size and shape can take any kind of medicine with equal readiness irrespective of the result which will ensue when the materials within the containers begin to operate. Applying this crude metaphor to plants and animals, in the one case, the germ cells, however they differ in factorial composition, come in the same cytoplasmic envelope; in the other, the cytoplasm as well as the genes may differ.

It has already been shown that, in particular cases, there is a selective action when pollen from different plants is applied to a stigma at the same time. East (1919) has demonstrated that in a mixture of compatible and incompatible pollen placed on the stigmas of self-sterile *Nicotianas* the compatible pollen alone functions. Two experiments were devised to test this. Small numbers of pollen grains were counted out under a microscope and placed on stigmas known to be receptive to that kind of pollen. The stigmas were then covered with a large amount of incompatible pollen. The former only was able to fertilize as shown by the results from eight such mixed pollinations which

in no case produced a greater number of seeds than the number of compatible pollen grains applied. In the other experiment a constant white-flowered self-sterile plant was pollinated with its own pollen. Several hours later pollen of a red-flowered plant was applied. Abundant seed was secured and when grown red-flowered cross-fertilized plants only were found. In both these cases there was complete selective fertilization in favor of the pollen from dissimilar plants.

According to the results to be reported here a directly reversed effect is obtained from mixed pollinations in maize. In many trials the plant's own pollen has been more efficient in accomplishing fertilization than that from other individuals which differ only in minor features. This same pollen which is less effective when in competition with the plant's own kind of pollen is fully able to function when not applied in mixtures. The results are remarkable in view of the notable advantages which cross-fertilization gives to the immediately resulting seeds and the plants grown from them. The material used consisted largely of self-fertilized strains which had been brought to uniformity and constancy and were considerably reduced in size and vigor. In this material crossing is known to increase the weight of seed within the same inflorescences as much as 50 per cent. in some cases. This greater amount of material is laid down both in the embryo and endosperm, and is apparent in the greater size of the seeds which show a higher specific gravity together with more rapid maturation indicated by their lower water content at the close of the growing period. A large series of mixed pollinations show the ability of the cross-fertilized seeds to germinate better by an average of 16 per cent. The resulting plants start to grow sooner, develop faster, mature in a shorter time and at the end far surpass their self-pollinated sibs. The amount of heterosis shown by maize is possibly greater than that displayed by any other plants in intra-specific crosses. Production of grain has been advanced on an average of 180 per cent., height of plant 27, length of pistillate spike 29, number of rows of spikelets 5 and number of nodes 6 per cent. in a study of a large number of crosses between inbred strains (Jones, 1918).

In spite of these great immediate advantages to be secured the

plants manifest a decided preference for their own kind of pollen. This is a result which would have surprised students of flower pollination in Darwin's time but which, as I shall attempt to point out later, is in agreement with other results from biological investigation.

METHODS OF CARRYING ON THE EXPERIMENTS.

In an investigation in which it was desired to compare the chemical composition of seeds of maize having different genetic constitution but produced under as nearly identical conditions as possible advantage was taken of endosperm characters to enable proper classification of seeds produced in the same inflorescences. For example, two kinds of pollen carrying yellow endosperm color and white endosperm color were mixed together and applied to a plant which normally produces uncolored seeds. The resulting yellow and white seeds were distributed at random on the pistillate spikes and were as nearly comparable in respect to external and nutritional factors as it is possible to obtain. It was found that such pollen mixtures could be applied to both strains furnishing the pollen and the two kinds of seeds easily distinguished. On the white-seeded strain the seeds resulting from the plant's own pollen were white and the cross-fertilized seeds yellow. On the yellow-seeded plants the self-pollinated endosperms were dark yellow while the crossed seeds were distinctly lighter in color, in most cases they had a white cap, and were as a rule readily separable.

Attention is directed to the fact that the material used for these mixed pollinations consisted largely of inbred strains which had been reduced to uniformity and constancy so that the genetical differences between the two kinds of seeds sharply differentiated them, much more than in ordinary cross-pollinated varieties of this plant in which the yellow color is usually variable due to more than one factor for this color and various modifying conditions such as the consistency of the endosperm in respect to corneous and floury starch.

A number of pairs of plants were treated in this way by mixing their pollen and applying to both types. After harvesting it was realized that here was an excellent method of determining

whether or not any selective action was shown by the plant's own pollen as compared to that from a plant of somewhat different type. If one member of the pair of plants which furnished the pollen for the mixture is designated A and the other B, the two kinds of seeds grown on A plants are $A \times A$, self-fertilized, and $A \times B$, cross-fertilized; on the B plants $B \times A$ cross-fertilized and $B \times B$ self-fertilized. Since the same pollen mixture is applied to both, the ratio of the seeds resulting from A pollen to the seeds resulting from B pollen, on A plants, should be the same as the ratio of the seeds resulting from A and from B pollen on B plants. In other words, the numbers form a proportion which, irrespective of the relative amounts of A and B pollen in the mixture, should be a perfect proportion within the limits of random sampling if fertilization takes place at random. The end terms of the proportion comprise the self-pollinated seeds and the middle terms the reciprocally cross-pollinated seeds. If a true proportion is obtained the products of the end terms should naturally equal the products of the middle terms. If they do not the deviation is either in an excess of cross-fertilized or of self-fertilized seeds indicating a selective action in one or the other direction.

The advantages of this method of attacking the problem are readily apparent. It is practically impossible to make up a mixture of large amounts of pollen in which the proportion of each is known. Either measuring or weighing the pollen is out of the question because maize pollen takes up moisture from the air rapidly and when any quantity is brought together it becomes aggregated into a flocculent mass. Moreover this pollen soon loses its viability so that even in case equal numbers of pollen grains could be had there would be no proof that there were equal numbers of functional pollen grains in the mixture.

The method of reciprocal application and arranging the results in the form of a proportion automatically overcomes all these difficulties and the experiment is as simple as could be devised. An attempt was made to have as nearly equal quantities of pollen as possible by measuring out the two kinds roughly. But in many cases the results showed that one kind of pollen was far more effective than the other. This, however, does not destroy

the value of the figures as it is the relative efficiency of each type of pollen, when it is applied to its own and to foreign stigmas, that is being investigated.

In the preliminary experiments there were 18 pairs of plants which produced seed as the result of the application of mixed pollen. When these were counted and the deviations of the proportions found from the closest perfect proportions were obtained there were 12 mixtures which showed a deviation in favor of the self-fertilized seeds and 6 in the opposite direction. The results as a whole showed a tendency to favor the plant's own pollen. This was somewhat unexpected so that it was considered worth while to investigate the matter more fully.

The inbred material was so reduced in growth that the number of seeds produced on one plant was not large enough to give the results much weight. It was therefore decided to pollinate a number of plants of two different self-fertilized strains with the same mixture. Most of the strains used had been self-pollinated for six generations or more, some as many as ten, so that the plants within one strain were practically identical in hereditary constitution. Pollen was collected from about the same number of plants as the pollen was applied to. The two lots of pollen were put together in a paper sack and thoroughly mixed by shaking. This mixture was then applied to plants of the two strains which supplied the pollen. It was desired to have from ten to fifteen plants in each of the paired strains so as to give from 1,000 to 2,000 seeds in each of the two parts of the proportions but the flowers were not always ready at the right time and some pollinations were failures for a variety of reasons so that not as large numbers as desired were secured in every case.

Every effort was made to prevent the entrance of undesired pollen. The technique has been described and the amount of experimental error due to contamination to be expected in artificial pollination of maize has been considered previously (East and Hayes, 1911). In the course of these experiments the effects of extraneous pollen were seen in very small numbers compared to the total number. This source of error could be detected in the seeds when colors or other characters differing from either of the strains used were brought in by the undesired

pollen. In a total of 63,000 seeds produced in these experiments only about 30 illegitimate seeds were observed. A larger number of contaminations, however, would have the same appearance as the legitimate seeds and so could not be detected but giving a reasonable allowance to this source of unreliability the results could not be appreciably altered. Moreover the error of this kind is never all in one direction so that in the main it can be disregarded.

The accuracy with which the seeds have been classified deserves particular attention. Strains were selected to be used which gave sharp differences between self-fertilized and cross-fertilized seeds and in most cases separation was made very satisfactorily. In a few mixtures there was some doubt and in two experiments the seeds on the yellow endosperm plants could not be distinguished. In these two cases the seeds were planted and classification was made with the mature plants. Also in all the other mixtures involving yellow and white endosperms a sample was taken, after all the seeds of one class were mixed together, and grown to determine the per cent. of error in separating the seeds. Since the self-fertilized seeds give small inbred plants, pure for yellow or white color, while the cross-fertilized seeds produce large vigorous hybrids segregating into yellow and white seeds, classification of the mature plants can be made without the least doubt. However, it should be noted that the better germination and greater vigor give the advantage to the cross-fertilized classes in every case if there is any difference. About 120 plants in each lot were grown and the per cent. of error obtained was used to calculate the total amount of error if all the seeds had been grown. Since the numbers of seed ran up into the thousands it was impossible to grow all of them. The figures showing the per cent. of errors found are arranged in Table III. Only in one case is the number of wrongly classified individuals above 3 per cent. In 25 out of 44 lots no faulty separations were discovered. In the remaining cases the misplaced seeds tend to balance each other so that this source of doubt can be largely removed. Calculating the figures without regard to the error of classification gives practically the same results as when this is taken into consideration.

Different characters were used in other mixed pollinations which permitted even more positive classification than the single character difference of yellow and white endosperms. Plants with yellow sweet seeds were paired with white starchy plants. Each strain brought in a dominant character. White starchy plants of the pop or *Zea mays everta* type were used because the clear corneous endosperm differentiates very clearly between yellow and white. In the reciprocal cross the smooth opaque seeds show up plainly contrasted with the wrinkled translucent seeds. Similarly purple sweet and white starchy types were paired. In a few cases the purple crossed seeds were not as distinct as could be desired, but the error is certainly small. These latter mixtures were made the past season and no plants have been grown to test the reliability of their separation but the writer is confident that the number of wrongly classified seeds, if any, is not sufficient to alter the results appreciably.

In many inflorescences a few seeds were found which had failed to reach a stage of development so that they could be classified. Seeds at the tips of the spikes and where the seeds were closely crowded were abortive. This introduces another source of unreliability, that of selective elimination of zygotes. Since crossing gives to seeds of maize an enormous advantage in development, it can confidently be expected that as a rule more of the self-pollinated seeds will be found among the abortions than cross-pollinated. What we are attempting to study is the relative fertilizing efficiency of different types of pollen grains. But one can only arrive at this by counting the zygotes sometime after fertilization has taken place. In the meantime a differential destruction of zygotes may have taken place. This effect must be considered in any organisms employed but because of the short time which elapses between fertilization and the maturation of the seeds, and from the fact that they develop in an exceedingly favorable and uniform environment, maize is the very best material the writer can think of in which this problem can be attacked, especially when the numbers which can be obtained are taken into consideration. All animals and those plants which do not show zenia in the seeds have the objection that a comparatively long time elapses between fertilization and suffi-

cient maturity to permit classification. In plants many cases are known in which there is a selective elimination of certain classes of individuals due to a lesser germination and unequal ability to grow. In *Drosophila* (Hyde, 1914) crossing does not influence the number of eggs laid but markedly regulates the per cent. that hatch. Therefore the error from this source always tends to show an apparent deviation in favor of cross-fertilization. In maize where the seed progenies can be used this differentiating effect is at a minimum and probably is not sufficient to affect the numbers appreciably but since the tendency is in the opposite direction to the results which have been obtained the data are even more convincing.

TABLE I.

CHARACTERS OF THE SEEDS AND PEDIGREE NUMBERS OF THE INBRED STRAINS AND FIRST GENERATION HYBRIDS USED IN THE MIXED POLLINATIONS.

Pollen Mixture Number.	Characters of the Seeds of		Pedigree Number of	
	A Strain.	B. Strain.	A Strain.	B. Strain.
1.....	Yellow Starchy	White Starchy	1-9-1-2-4-6-7-5-6-2-1	10-4-8-3-5-3-4-8-2-1
2.....	" "	" "	1-9-1-2-4-6-7-5-3-2-1	10-4-8-3-5-3-4-5-2-1
3.....	" "	" "	14-4-6-16-2-12-22	21-13-9-7-57-11
4.....	" "	" "	14-4-6-16-2-12-22	21-13-9-7-57-11
5.....	" "	" "	14-4-6-16-2-12-22	21-13-9-7-57-11
6.....	" "	" "	14-30-4-3-7-11-10	21-13-9-7-57-11
7.....	" "	" "	14-30-4-3-7-11-10	20-4-25-47-4
8.....	" "	" "	14-4-6-16-2-12-22	20-8-5-35-20
9.....	" "	" "	1-9-1-2-4-6-7-5-6-2-1-1	20-4-25-47-4
10.....	" "	" "	(1-6-1-3 × 1-9-1-2) F ₁	(20-8 × 21-13) F ₁
11.....	" "	" "	(1-6-1-3 × 1-7-1-1) F ₁	(20-8 × 21-13) F ₁
12.....	" "	" "	(1-7-1-2 × 1-6-1-3) F ₁	(21-13 × 20-8) F ₁
13.....	Yellow Sweet	White Starchy	146-1-1	65-8-2-2-6-5-3-2-1-1-1
14.....	" "	" "	146-1-1	65-8-2-2-6-5-3-2-1-1-1
15.....	Yellow and White Sweet	" "	(126-1-1-1 × 77) F ₁	117-3-1-1
16.....	" " " "	" "	(126-1-1-1 × 77) F ₁	117-1-1-1
17.....	Purple Sweet	White Starchy	76-2-2-1-1	117-1-1-1
18.....	" "	" "	76-2-1-2-1	117-1-1-1
19.....	" "	" "	76-2-1-2-1	20-4-25-47-24-1
20.....	" "	" "	76-2-2-1-1	20-4-25-47-24-1
	C Strain		C Strain	
1.....	Yellow Starchy		1-6-1-3-4-4-4-2-4-2-5	
19.....	White Sweet		77-2-1-1-1	
20.....	White Starchy		117-1-1-1	

MATERIALS USED AND PRESENTATION OF THE RESULTS.

The figures obtained from the preliminary experiments in which pairs of single plants only were used are not given here because the numbers are too low to give the results much value and for fear of making this report too bulky. It is sufficient to remember that the data taken together indicated a slight selective action favoring the plant's own pollen.

TABLE II.

SUMMARY OF ALL THE POLLEN MIXTURES GIVING THE TOTAL NUMBER OF SEEDS, THE NUMBERS IN EACH CLASS FORMING PROPORTIONS, THE DEVIATIONS OF WHICH, EXPRESSED AS PER CENT., FROM THE CLOSEST TRUE PROPORTIONS, ARE ALMOST WHOLLY IN FAVOR OF THE PLANT'S OWN POLLEN. P IS THE PROBABILITY THAT THESE DEVIATIONS ARE DUE TO THE DIFFERENCES OF RANDOM SAMPLING.

Pollen Mixture Number.	Total Number of Seeds.	A × A.	A × B.	B × A.	B × B.	Deviation from True Proportion, Per Cent.	χ^2 .	P.
1	3,430	1,738	46	1,602	44	+ .045	.027	.994
2	5,636	2,133	145	3,080	278	+ .955	7.572	.063
3	1,362	229	14	770	349	+12.715	146.196	.000
4	3,344	710	126	1,856	652	+ 5.465	61.203	.000
5	1,956	589	6	1,290	71	+ 2.105	28.718	.000
6	424	40	71	187	126	-11.850	23.858	.000
7	3,459	23	89	1,507	1,840	-12.245	235.357	.000
8	7,783	2,185	956	2,619	2,023	+ 6.570	144.108	.000
9	8,729	2,550	1,288	2,922	1,969	+ 3.350	42.061	.000
10	5,408	1,084	1,154	997	2,173	+ 8.495	162.687	.000
11	3,314	448	264	1,505	1,097	+ 2.540	8.937	.030
12	3,561	1,724	719	749	369	+ 1.790	5.313	.053
13	736	185	391	95	65	-13.625	55.051	.000
14	792	424	150	156	62	+ 1.155	.533	.894
15	3,168	2,609	47	14	498	+47.750	2,889.561	.000
16	2,224	723	8	74	1,419	+46.975	1,965.981	.000
17	1,410	1,303	3	4	100	+47.960	1,298.993	.000
18	1,599	4	21	1	1,573	+ 7.970	137.532	.000
19	2,606	528	392	343	1,343	+18.525	376.394	.000
20	2,753	897	77	1,174	605	+13.050	283.002	.000
	63,694							

Of those experiments in which pollen was applied to several plants of the same type 20 pollen mixtures in all have been made.

Each mixture is given a number and the seed characters of the materials used and their pedigree numbers are given in Table I. The first number in the pedigree designates the variety from which the inbred strains were derived. The following numbers indicate the progenitors in the successive self-fertilized generations. The total number of units in the pedigree number less one, show the number of generations the material had been self-fertilized at the time the pollinations were made. The inbred strains used in these experiments are as follows:

1. Several distinct strains from a yellow dent variety originally obtained in Illinois and known as Chester's Leaming, self-fertilized ten or more years.

10. A strain with white flourey seeds with no traces of corneous starch, self-fertilized nine generations.

14. Two distinct strains from a yellow dent variety from Connecticut known as Stadtmueller's Leaming, selected for high protein content during six generations of self-fertilization.

20. Two distinct strains from a white dent variety originally selected for high protein at the Illinois Exper. Station and further selected during four generations of self-fertilization.

21. One strain from same source as above but selected for low protein in field pollinated cultures and during five generations of self-fertilization.

65. A small, white, round-seeded strain from a variety of popcorn, *Zea mays everta*, self-fertilized ten times and characterized by clear corneous starch.

76. Two similar strains from a sweet variety of latent flint type having purple aleurone and known as Black Mexican, self-fertilized four years.

77. From a sweet variety of latent dent type with deeply wrinkled white seeds known as Evergreen and self-fertilized four generations.

126. From a small, early maturing, yellow, sweet variety of latent flint type with dark yellow kernels, known as Golden Bantam, and self-fertilized three generations.

117. From a variety of popcorn with sharp pointed seeds having clear corneous endosperm, self-fertilized three times.

146. From a yellow, sweet variety, Golden Bantam, of different

source than 126, and somewhat different in type, self-fertilized two years.

Mixtures number 1 to 9 inclusive comprise various inbred strains with yellow starchy and white starchy endosperm. Some of these strains have been described previously (Jones, 1918) and all show marked heterosis in the crossed seeds and in the resulting first generation hybrid plants. A sample of all the different lots of seed secured in these mixtures have been grown to test the accuracy of classification. Pollen mixtures number 10 to 12 are not from inbred strains but from first generation hybrids, one having all yellow seeds the other all white. They were of such a constitution that the second crossing gave still more increase in vigor although not as great as the stimulus following the first cross. The plants being vigorous a large amount of seed was obtained from a few plants. It was desired to know whether the same selective action would be shown by vigorous plants with segregating gametes as contrasted with non-vigorous plants whose gametes were all alike. Classification of the seed was easily carried out and the per cent. of error when tested was found to be quite low. Mixtures 13 and 14 involved yellow sweet in one strain and white starchy endosperm in the other. One dominant factor was carried by each so that differentiation was perfectly distinct in the reciprocal applications. In mixtures 15 and 16 it was intended to make use of the same characters as in the two preceding numbers. The plants which were supposed to be inbred individuals of a yellow sweet strain, and so labeled, when grown in the field were seen to be too vigorous for this material as it had behaved in previous years. The plants were all alike, however, in this respect. It was suspected that this was a lot of first generation hybrids instead of plants from self-fertilized seed. As some crosses had been made with plants of this line the previous season it is now certain that in this instance the seed was not properly labeled at harvest and so was planted for self-fertilized seed when in reality it was all cross-fertilized. As no other plants were available at the time they were needed these were used. Several self-pollinations were made at the same time to show what the seed characters were of this undoubted hybrid. At maturity it definitely showed itself

to be a hybrid as all the ears were segregating for yellow and white sweet seeds. It therefore had certainly been crossed with a white sweet inbred strain the year before as other pollinations of this sort were made at that time. The yellow color of the endosperm was not sufficiently diluted to cause the seed to be suspicioned before planting.

Since the plants were segregating for yellow and white it was not material which would have been used ordinarily for mixing with a white starchy strain. The effects of the starchy-carrying pollen showed up all right among the all sweet seeds but the reciprocal cross-pollination showed only the yellow cross-fertilized seeds. The white cross-fertilized seeds of course could not be distinguished from the self-fertilized seeds. But since half of the pollen grains carried yellow and half of them white the number of yellow seeds can be doubled to give the total number of cross-pollinated seeds and the assumed number of white cross-fertilized seeds subtracted from the white seeds. This increases the error from random sampling somewhat but since the number of yellow seeds is very low in comparison with the white in these mixtures, the data are reliable in view of the great selective action shown in these two mixtures. The fact that the yellow color in this material was a unit factor difference and that there were equal number of pollen grains carrying yellow and white is proven by the self-fertilized ears produced by the hybrid which gave very good 3 to 1 ratios (one ear counted gave 318 yellow and 97 white seeds). Furthermore the starchy crossed seeds produced on the hybrid plants were of two kinds, yellow and white, and were produced in equal numbers (actual numbers: 28 yellow and 27 white. Since the ovules were segregating equally and the self-fertilized seeds gave the mono-hybrid ratio the pollen grains must have carried the two colors in equal amount.

In the last four mixtures the characters, purple sweet and white starchy, were used. In numbers 17 and 18 the plants were not productive and the numbers of seeds are low. Also the classification of purple starchy cross-fertilized seeds and white starchy self-fertilized was not as sure as in the other mixtures. In the last two mixtures satisfactory numbers were obtained and the differentiation was clear-cut on both sides.

The pollen mixtures 1, 19 and 20 were also applied to a third strain distinct from either of the two used in supplying the pollen. The resulting two lots of seed in each case were both cross-fertilized and probably showed hybrid vigor in the seed in about equal amount. Heterosis is clearly apparent in the plants of similar crosses involving the same or closely related material and is approximately equal.

The data from each pollen mixture are presented in the form of an appendix. Since the arrangement is the same in all the tables these are given in the simplest form possible and a description of one applies to all. In the tables the headings for the four different classes of seed are $A \times A$, $A \times B$, $B \times A$, $B \times B$ and in the three mixtures 1, 19 and 20 there are in addition the out-crossed seeds $C \times A$, and $C \times B$. In every case the pistillate parent is given first. These headings when expanded in detail are as follows:

$A \times A$ = Self-fertilized seeds from color-carrying pollen.					
$A \times B$ = Cross	"	"	"	non-color	"
$B \times A$ = Cross	"	"	"	color	"
$B \times B$ = Self	"	"	"	non-color	"
$C \times A$ = Out-crossed	"	"	"	color	"
$C \times A$ = Out	"	"	"	non-color	"

On every plant there are two kinds of seeds indicating the relative fertilizing efficiency of the two kinds of pollen in the mixture used. The number of seeds from individual plants are given in the tables under their respective headings, $A \times A$ and $A \times B$ seed from the same inflorescence and $B \times A$ and $B \times B$ seeds from another and $C \times A$ and $C \times B$ from a third. The total number of seeds is summed up below the line. Next to that is given the per cent. of error found upon testing the accuracy of the classification. This figure is used to calculate the amount of error based upon all the seeds obtained. Not more than about 125 seeds were grown in each lot except in two cases as shown in Table III. The calculated amount of wrongly classified seeds is given on the line below the per cent. of error, then follows the corrected numbers after the proper additions and subtractions have been made. After that is placed the observed proportion

of the two kinds of seed in each member of the pair stated as per cent. The closest perfect proportion is calculated from this, based on the results from the A and B plants but not from the C plants, and this subtracted from the actual proportion found gives the deviation in per cent. which appears in the last line. This description applies to mixtures 1 to 12 inclusive. Of the remainder no correction for misplaced seeds is made.

TABLE III.

NUMBER OF PLANTS GROWN AND THE PER CENT. OF WRONGLY CLASSIFIED SEEDS IN THE POLLEN MIXTURES INVOLVING YELLOW AND WHITE, STARCHY ENDOSPERM.

Pollen Number. Mixture	A. × A.		A. × B.		B. × A.		B. × B.	
	Number.	Per Cent.	Number.	Per Cent.	Number.	Per Cent.	Number.	Per Cent.
1.	414	1.21	26	7.69	107	0	27	22.22
2.	483	2.69	50	8.00	115	0	128	2.34
3.	105	0	12	0	119	0	114	1.75
4.	116	0	101	.99	120	.83	114	1.72
5.	110	0	6	0	120	0	56	0
6.	40	—	71	—	112	0	76	2.63
7.	23	—	89	—	119	0	118	0
8.	119	0	118	0	120	0	118	1.69
9.	118	2.54	118	2.54	120	1.67	113	0
10.	117	1.71	118	0	118	1.69	117	1.71
11.	114	.88	119	0	123	0	120	0
12.	116	.86	117	0	116	0	116	0

The total number of seeds, the numbers in each class and the deviations in per cent. from the closest perfect proportion for the 20 pollen mixtures are summarized in Table II. The deviation, if it is an excess of self-fertilized seeds, is given as plus and, if the opposite, as minus. The deviations can range from + 50.0 to - 50.0 per cent. The extremes indicate complete non-functioning of each kind of pollen on one set of plants and exclusive functioning on the other.

Altogether the number of seeds amounts to 63,694. Large numbers are, of course, necessary to be convincing in any experiment on selective fertilization as such investigations are largely studies in sampling. Mixtures number 6, 7, 13, 17 and 18 are less reliable than the others, because they have rather small numbers from either the A or B plants, less than 200 in each case. Like the chain with the weak link the value of each set of figures

is dependent upon the numbers in the less populous half of the proportion.

Some of the mixtures have over one thousand individuals in each of the four classes. Since the experimental error is low and not all in one direction as has been shown and the selective elimination of zygotes tends to obscure the result which has been obtained, such figures as these can not be gainsayed. Of the 20 mixed pollinations 17 show a deviation indicating a selective action in favor of the plants' own pollen. While three of the mixtures show the opposite effect. These three are all low in number of individuals on one or the other side of the proportion. Mixtures 6 and 7 could not be classified by the seeds on the yellow seeded plants so consequently the progenies were grown and classified at maturity. This brings in other sources of error—differential germination and competition between plants which are weak with those that are vigorous—which certainly tend to result to the apparent advantage of cross-fertilization. Two of the deviations showing an excess of self-fertilized individuals are not significant when compared to the allowable differences from random sampling but all the others are. Therefore the conclusion is ineluctable that in maize the plant's own pollen is more effective in consummating fertilization than pollen from plants of only slightly different construction. This selective action is shown even though the foreign pollen is perfectly capable of fertilizing the plants when not acting in competition with the plant's own kind of pollen as has been definitely proven. Mixtures number 15, 16 and 17 show 47 out of a possible 50 per cent. deviation, almost complete non-functioning of the unfamiliar pollen. Numbers 15 and 16 include the first generation hybrids in which some calculations had to be made to allow for segregation but these are perfectly justifiable adjustments and the results can be discounted but very little. Mixture 17 is low in numbers having only 104 seeds on the B plants of which 4 are cross-fertilized. But observe the result when this same mixture is applied to the A plants. Here only 3 cross-fertilized seeds are to be found among 1,303 self-fertilized seeds. Surely there is some powerful action working to hold back the unfamiliar pollen. Mixtures number 3, 4, 8, 10, 19 and 20 are by them-

selves convincing as in these the numbers are large, the differentiation of the seeds in both groups is precise and the deviations clearly show the superiority of self-pollination.

The data from pollen mixture number 1 have been published previously (Jones, 1918) although at that time plants had not been grown to test the error in separating the seeds. The greatest number of mistakes of classification of any of the mixtures were made in this lot and the deviation is now well within the limits of random sampling. The data are included here to make this report complete. In the previous publication the probable error used was the familiar formula used for Mendelian ratios. The determinations applied to each half of the proportion alone. Since the ratio on each of the paired plants is dependent upon the ratio on the other, it seems to the writer now that the use of this method of calculating the probable error in connection with this particular problem is wrong.

The method of calculating the significance of the figures as used here is that proposed by Elderton (1901) and is in general use in presenting genetic data. It is obtained in the following manner. The deviations of the terms in the actual proportion found from the closest perfect proportion as calculated are squared and divided by the terms of the perfect proportion. Their sum gives a value χ^2 , which by use of convenient tables calculated by Elderton, gives a probability value varying from 0 to 1 proportional to the goodness of fit.

The calculations must be based on the actual numbers of seeds obtained and not on the percentages. To obtain a perfect proportion from which the deviations of the numbers found will be the smallest in the four terms it is necessary to balance the figures so that the same number of individuals are represented on the A and B plants. This is done by reducing the number of seeds of the greater and increasing the lesser keeping the ratios the same, of course. The deviations of the proportions, balanced in this way, from the closest perfect proportions are then used to obtain the probability value in the way described above. The same result is obtained more quickly by calculating the value of χ^2 from the percentages and multiplying this figure by one half the total number of seeds.

With four terms the values of χ^2 greater than 18 have no probability out to three decimal places. Since very much greater numbers were secured in most cases, as shown in Table II., the deviations are clearly not due to the differences of random sampling alone if the application of this method of calculation is justifiable. The writer is not perfectly sure that it is because it should be noted that the theoretical proportion is calculated directly from the results found, that is, there is no possible way of knowing the real amounts of the two kinds of functional pollen contained in the mixtures. Moreover the probable error does not take into consideration the corrections which are made for the mistakes of classification found in a sample drawn from each lot.

There may be a selective action when the pollen is applied to one plant but not to the other or the action may be reversed. All that is measured is the combined effect if both are in the same direction or the excess of one over the other if in opposite directions. It seems reasonable to suppose, however, that the selective fertilization is approximately the same on both members of the pair as a large number of mixed pollinations are available made with many different types of plants and the majority give the same result. If this were always true, however, the ratio obtained by out-crossing the mixture onto a distinct strain should not deviate from the ratio of the closest perfect proportion calculated from the figures of the reciprocal crosses beyond allowable limits. That is, the ratio obtained from the out-crossed seeds is supposed to represent very nearly the actual ratio of effective pollen in the mixture since both kinds are more nearly on the same footing. In the three experiments, Nos. 1, 19 and 20 in which such out-crossed seeds were obtained, the deviation is even greater in two cases than that from the reciprocal applications. In pollen mixture number 1 all the deviations are small and probably without significance.

Since the results are convincing when considered without a probable error, it is not necessary to lay much stress on the method of its calculation at this time. Considering the data altogether, magnifying the actual experimental error to its fullest extent, and taking a common-sense view of the allowances to be

made for variations inherent in a problem of this kind the conclusion can be no other than that these plants manifest a definite receptiveness to their own pollen, discriminating against foreign pollen even though it comes from plants only slightly differentiated from them, both of which might easily be descended from the same individual at no very distant period back. This selective action is shown by plants of weak growth or full vigor, whether each strain descended from a line of similar ancestors or whether its immediate parents were diverse and, finally, irrespective of the gametes being alike or unlike in germinal contents. The one significant feature in common in all these experiments is the fact that the cytoplasm which surrounds the male nuclei and which makes up the vehicle that carries them to the egg cells is alike for the gametes of any one type of plant whether this plant is homozygous or heterozygous and in self-fertilization this cytoplasm is the same as the medium in which the pollen fulfills its function. This points very strongly to the probability that the differential effect is due to the rate of pollen-tube growth although it may be determined after the male nuclei are brought to the egg.

The average weight of the seeds in the different classes of all the pollen mixtures is given in Table IV. with the increase in weight of the cross-fertilized over the self-fertilized seeds. Expressed as per cent. these figures permit an estimation of the comparative amount of heterosis shown in the crosses. In fact this is one of the best means of measuring the stimulation of heterozygosis as the environmental differences are reduced to a minimum. It has not been definitely proven that there exists a correlation between the amount of heterosis in the seeds and that shown by the resulting plants grown from those seeds but the indications are that there is a close relation between the two. Since hybrid vigor is roughly proportional to the germinal differences in the two forms united it can be determined whether or not there is a relation between the diversity of the plants used in the several pollen mixtures and the degree of preference shown by those plants to their own kind of pollen. Table V. shows that the coefficient of correlation between the average increase in weight of seeds and the deviation in favor of self-fertilization, both stated

TABLE IV.

THE AMOUNT OF HETEROSIS SHOWN BY EACH CROSS-POLLINATION IN THE INCREASE IN AVERAGE WEIGHT OF SEEDS COMPARED TO THE SELF-POLLINATED SEEDS GROWN IN THE SAME INFLORESCENCES.

Pollen Mixture Number.	Average Weight of Seeds in Centigrams.						Per Cent. Increase.		
	A × A.	A × B.	Increase.	B × A.	B × B.	Increase.	A.	B.	Ave.
1	13.8	18.1	4.3	18.0	15.7	2.3	30.4	14.6	22.5
2	12.2	17.1	4.9	17.6	14.3	3.3	40.0	23.0	31.5
3	29.3	35.5	6.2	39.8	35.7	4.1	21.2	11.5	16.4
4	26.9	31.4	4.5	34.2	29.3	4.9	16.7	16.7	16.7
5	30.0	47.6 ¹	17.6	38.3	34.8	3.5	58.7	10.1	34.4
6	—	—	—	36.7	34.7	2.0	—	5.8	5.8
7	—	—	—	19.6	16.3	3.3	—	20.2	20.2
8	25.8	30.6	4.8	23.8	22.5	1.3	18.6	5.8	12.2
9	12.0	15.8	3.8	15.7	13.2	2.5	31.7	18.9	25.3
10	32.2	33.4	1.2	32.5	30.9	1.6	3.7	5.2	4.5
11	27.5	28.6	1.1	34.5	32.8	1.7	4.0	5.2	4.6
12	32.4	32.8	.4	37.4	34.3	3.1	1.2	9.0	5.1
13	25.7	29.3	3.6	16.4	14.8	1.6	14.0	10.8	12.4
14	20.2	22.1	1.9	13.9	12.9	1.0	9.4	7.8	8.6
15	24.2	35.1	10.9	12.9	11.7	1.2	45.0	10.3	27.7
16	32.0	40.0 ¹	8.0	11.4	9.6	1.8	25.0	18.8	21.9
17	14.3	20.0	5.7	15.0	10.2	4.8	39.9	47.1	43.5
18	25.0	23.8 ¹	-1.2	10.0 ¹	8.2	1.8	-4.8	22.0	8.6
19	15.7 ¹	22.2	6.5	16.6 ¹	15.6	1.0	41.4	6.4	23.9
20	14.3	17.1	2.8	15.9	15.3	.6	19.6	3.9	11.8

in per cent., is $+.496 \pm .093$. Although the numbers are scanty there is a significant relation between the two. This means that the more unlike the plants are the greater the distinction that is made between the two kinds of pollen. *In proportion as the cross-fertilization benefits the immediate progeny in its development the less effective is that pollen in accomplishing the union.*

The same method of experimentation was applied to another plant, the garden tomato, *Lycopersicum esculentum* Mill. Advantage was taken of plant characters such that the seedlings could be distinguished in both reciprocal applications. Pollen from a variety with entire leaves with a tall habit of growth was mixed with pollen from a dwarf variety with normal, serrate leaves. Tall stature and normal leaves are dominant so that the cross-fertilized and self-fertilized seedlings from one variety were

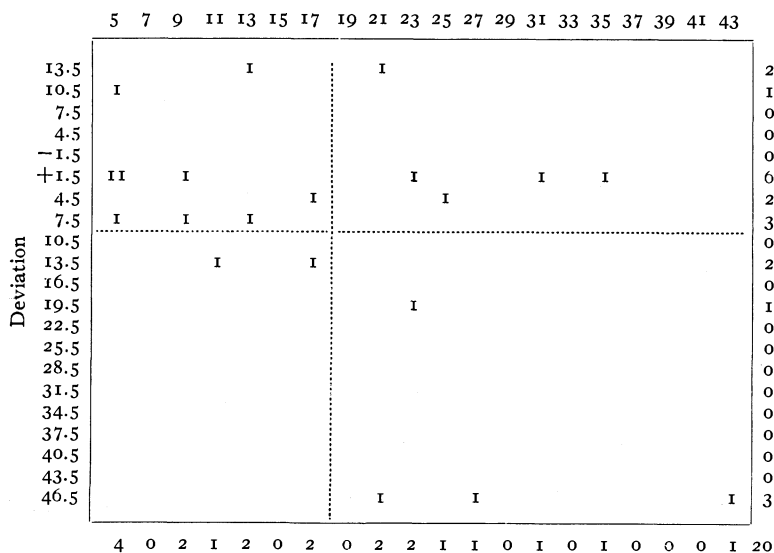
¹ Number of seeds too few to make averages reliable.

visible because of differences in leaf formation and in the other variety by habit of growth. Dwarf plants are characteristically shorter and more compact in stems and leaves which gives them a distinct appearance. The plants used were grown from unpedigreed seed but the tomato is usually self-fertilized and the varieties employed were tested and found to come true to type. Two experiments were made and the results from these are given as pollen mixtures number 21 and 22 in the appendix. The plants from individual fruits are grown separately. The total numbers in the two mixtures are 340 and 272. Differentiation was sharp in the seedling stage in the A lots with either serrate or entire leaves. In the B lots the presence of tall and dwarf seedlings could be easily seen but not all of them could be separated as positively as could be desired. The plants were therefore set in the field and classified after they had grown about two months. They were set out too late to make a satisfactory growth and even at the end of the season classification was not made with certainty in every case.

TABLE V.

CORRELATION BETWEEN THE AMOUNT OF HETEROSIS, SHOWN BY THE PER CENT.
INCREASE IN WEIGHT OF CROSSED SEEDS, AND THE SELECTIVE ACTION IN
FAVOR OF THE PLANTS' OWN POLLEN. $r = +.496 \pm .093$.

Per Cent. Increase in Weight.



POLLEN MIXTURE NO. 1.

A × A.	A × B.	B × A.	B × B.	C × A.	C × B.
317	1	319	5	361	10
189	2	224	9	429	15
270	7	369	10	421	13
389	8	348	26	445	1
332	8	330	6		
260	1				
1,757	27	1,590	56	1,656	39
1.21	7.69	0	22.22	.39	6.66
21	2	0	12	6	3
1,738	46	1,602	44	1,653	42
97.420	2.580	97.330	2.670	97.520	2.480
97.375	2.625	97.375	2.625	97.375	2.625
+.045	-.045	-.045	+.045	+.145	-.145

POLLEN MIXTURE NO. 2.

A × A.	A × B.	B × A.	B × B.
236	11	359	42
206	9	353	20
188	15	380	42
141	3	230	15
194	8	372	39
237	11	214	12
232	11	286	36
167	7	265	19
151	3	273	31
188	8	341	29
245	7		
2,185	93	3,073	285
2.69	8.00	0	2.34
59	7	0	7
2,133	145	3,080	278
93.630	6.370	91.720	8.280
92.675	7.325	92.675	7.325
+.955	-.955	-.955	+.955

The results, taken as they stand, are the same as obtained from maize. There is a deviation favoring the plants' own pollen of 2.06 and 6.84 per cent., in the two cases with the probability values .907 and .082 respectively. In the first instance the difference can easily be due to random sampling, in the other the odds are strongly against such an explanation. In view of the fact that differences in germination of the seeds and viability of plants most certainly tend to decrease the proportion of self-fertilized individuals, the data have some value. They should be corroborated by larger numbers using other characters which can be more surely identified before they are as convincing as the results with maize.

POLLEN MIXTURE NO. 3.

A × A.	A × B.	B × A.	B × B.
23	3	90	41
54	7	114	28
27	0	27	26
34	1	15	20
18	0	97	56
43	2	73	19
19	0	76	52
4	0	27	8
7	1	50	3
		41	14
		66	55
		33	5
		22	11
		33	17
229	14	764	355
0	0	0	1.75
0	0	0	6
229	14	770	349
94.240	5.760	68.810	31.190
81.525	18.475	81.525	18.475
+12.715	-12.715	-12.715	+12.715

POLLEN MIXTURE NO. 4.

A × A.	A × B.	B × A.	B × B.
189	38	301	67
173	42	208	102
165	11	248	70
64	14	164	167
41	7	194	38
77	15	254	117
		213	38
		278	49
709	127	1,860	648
0	.99	.83	1.72
0	1	15	11
710	126	1,856	652
84.930	15.070	74.000	26.000
79.465	20.535	79.465	20.535
+5.465	-5.465	-5.465	+5.465

POLLEN MIXTURE NO. 5.

A × A.	A × B.	B × A.	B × B.
23	2	219	8
123	3	246	14
56	1	313	24
143	0	352	19
244	0	160	6
589	6	1,290	71
98.990	1.010	94.780	5.220
96.885	3.115	96.885	3.115
+2.105	-2.105	-2.105	+2.105

POLLEN MIXTURE NO. 6.

A × A.	A × B.	B × A.	B × B.
40	71	184	129
—	—	0	2.63
—	—	0	3
40	71	187	126
36.04	63.96	59.74	40.26
47.89	52.11	47.89	52.11
-11.85	+11.85	+11.85	-11.85

POLLEN MIXTURE NO. 7.

A × A.	A × B.	B × A.	B × B.
23	89	253	188
		104	189
		185	212
		84	117
		166	174
		115	139
		118	156
		123	190
		116	169
		109	158
		134	148
23	89	1,507	1,840
20.540	79.460	45.030	54.970
32.785	67.215	32.785	67.215
-12.245	+12.245	+12.245	-12.245

POLLEN MIXTURE NO. 8.

A × A.	A × B.	B × A.	B × B.
96	53	173	108
158	112	98	69
154	60	74	83
136	47	102	102
106	38	175	131
103	29	199	147
135	65	109	79
89	53	196	89
130	47	142	153
60	11	203	131
128	49	117	93
63	38	52	52
69	29	166	106
87	34	109	79
186	67	83	72
59	31	138	86
103	54	133	138
77	34	76	75
124	51	45	41
58	18	119	116
64	36	75	108
2,185	956	2,584	2,058
0	0	0	1.69
0	0	0	35
2,185	956	2,619	2,023
69.56	30.44	56.42	43.58
62.99	37.01	62.99	37.01
+6.57	-6.57	-6.57	+6.57

POLLEN MIXTURE No. 9.

A × A.	A × B.	B × A.	B × B.
277	149	209	115
116	69	173	99
168	69	264	156
135	78	188	114
151	52	160	101
78	30	210	142
128	71	172	134
254	102	191	160
286	147	166	112
208	97	195	134
185	88	196	107
113	55	175	108
208	117	207	105
277	130	128	97
		92	73
		123	76
		123	86
2,584	1,254	2,972	1,919
2.54	2.54	1.67	0
66	32	50	0
2,550	1,288	2,922	1,969
66.44	33.56	59.74	40.26
63.09	36.91	63.09	36.91
+3.35	-3.35	-3.35	+3.35

POLLEN MIXTURE No. 10.

A × A.	A × B.	B × A.	B × B.
344	331	189	397
200	272	195	447
284	250	189	522
275	282	233	399
		169	430
1,103	1,135	975	2,195
1.71	0	1.69	1.71
19	0	16	38
1,084	1,154	997	2,173
48.440	51.560	31.450	68.550
39.945	60.055	39.945	60.055
+8.495	-8.495	-8.495	+8.495

POLLEN MIXTURE No. 11.

A × A.	A × B.	B × A.	B × B.
67	57	209	130
385	203	290	199
		279	265
		386	191
		341	312
452	260	1,505	1,097
.88	0	0	0
4	0	0	0
448	264	1,505	1,097
62.92	37.08	57.84	42.16
60.38	39.62	60.38	39.62
+2.54	-2.54	-2.54	+2.54

POLLEN MIXTURE No. 12.

A×A.	A×B.	B×A.	B×B.
552	196	218	106
86	67	197	92
265	111	163	69
253	114	105	46
182	61	66	56
401	155		
1,739	704	749	369
.86	0	0	0
15	0	0	0
1,724	719	749	369
70.57	29.43	66.99	33.01
68.78	31.22	68.78	31.22
+1.79	-1.79	-1.79	+1.79

POLLEN MIXTURE No. 13.

A × A.	A × B.	B × A.	B × B.
37	52	14	11
17	87	13	18
11	16	17	11
28	86	6	4
50	91	31	8
42	59	14	13
185	391	95	65
32.120	67.880	59.370	40.630
45.745	54.255	45.745	54.255
-13.625	+13.625	+13.625	-13.625

POLLEN MIXTURE No. 14.

A×A.	A × B.	B × A.	B × B.
41	13	58	31
91	35	35	15
83	39	46	10
70	27	17	6
139	36		
424	150	156	62
73.870	26.130	71.560	28.440
72.715	27.285	72.715	27.285
+1.155	-1.155	-1.155	+1.155

POLLEN MIXTURE No. 15.

A×A.	A×B.	B×A.	B×B.
309	11	3	111
415	11	0	53
393	9	1	71
326	7	1	113
276	2	0	67
135	1	2	90
400	4		
355	2		
2,609	47	(7)	(505)
		14	498
98.23	1.77	2.73	97.27
50.48	49.52	50.48	49.52
+47.75	-47.75	-47.75	+47.75

POLLEN MIXTURE NO. 16.

A × A.	A × B.	B × A.	B × B.
285	5	12	355
187	1	8	350
251	2	8	408
		4	226
		5	117
723	8	(37)	(1,456)
		74	1,419
98.910	1.090	4.960	95.040
51.935	48.065	51.935	48.065
+46.975	-46.975	-46.975	+46.975

POLLEN MIXTURE NO. 17.

A × A.	A × B.	B × A.	B × B.
255	1	4	49
166	0	0	51
185	1		
222	0		
220	1		
255	0		
1,303	3	4	100
99.77	.23	3.85	96.15
51.81	48.19	51.81	48.19
+47.96	-47.96	-47.96	+47.96

POLLEN MIXTURE NO. 18.

A × A.	A × B.	B × A.	B × B.
3	5	1	368
1	16	0	432
		0	320
		0	336
		0	39
		0	78
4	21	1	1,573
16.00	84.00	0.06	99.94
8.03	91.97	8.03	91.97
+7.97	-7.97	-7.97	+7.97

POLLEN MIXTURE NO. 19.

A × A.	A × B.	B × A.	B × B.	C × A	C × B.
20	16	41	206	75	213
89	53	36	147	71	124
68	86	42	200	61	315
55	47	45	178		
69	39	29	150		
45	20	63	156		
52	37	30	88		
65	8	57	218		
65	86				
528	392	343	1,343	207	652
57.390	42.610	20.340	79.660	24.100	75.900
38.865	61.135	38.865	61.135	38.865	61.135
+18.525	-18.525	-18.525	+18.525	-14.765	+14.765

POLLEN MIXTURE NO. 20.

A × A.	A × B.	B × A.	B × B.	C × A.	C × B.
172	11	193	86	40	52
43	6	104	58	46	56
165	15	117	86	51	55
38	2	80	45	2	5
89	4	47	17		
103	8	128	52		
82	12	81	47		
19	0	94	60		
53	4	92	30		
33	0	99	43		
69	13	75	46		
31	2	64	35		
897	77	1,174	605	139	168
92.09	7.91	65.99	34.01	45.28	54.72
79.04	20.06	79.04	20.96	79.04	20.96
+13.05	-13.05	-13.05	+13.05	-33.76	+33.76

POLLEN MIXTURE NO. 21.

A × A.	A × B.	B × A.	B × B.
39	16	17	25
12	14	1	5
32	25	36	18
14	18		
97	73	54	48
57.06	42.94	52.94	47.06
55.00	45.00	55.00	45.00
+2.06	-2.06	-2.06	+2.06

POLLEN MIXTURE NO. 22.

A × A.	A × B.	B × A.	B × B.
7	22	13	17
10	27	7	27
107	103		
124	152	20	44
44.93	55.07	31.25	68.75
38.09	61.91	38.09	61.91
+6.84	-6.84	-6.84	+6.84

PREVIOUS INVESTIGATIONS ON SELECTIVE FERTILIZATION.

From the work of Kölreuter, Herbert, Gärtner, Darwin, Müller, Knuth and others we are familiar with the phenomenon of self-sterility in plants in which the individual's own pollen is wholly incapable of functioning on the plant by which it is produced although perfectly developed and able to fulfill its duties when brought to other plants of the same species. Numerous investigators have been giving attention to this problem in recent times. East and Park (1917) have made a noteworthy contribu-

tion to its solution and give a complete résumé of the work which has been done on this subject. They have been able to demonstrate that groups exist within which the individuals are all both self-sterile and cross-sterile, but any member of one group is perfectly fertile with any member of any other group. These investigators find that there are about 100 well-endorsed instances of self-sterility in plants scattered over some 35 families. Undoubtedly this discrimination is a means to promote cross-fertilization of approximately the same significance as floral contrivances, dichogamy and diœcism. Even though widespread in its occurrence self-sterility is a special adaptive process fulfilling a particular function. It is directly opposite in its effect to the results found in maize which shows no self-sterility of the type found in *Nicotiana* and other genera. At least no clear cases are known of maize pollen, which is unable to fertilize the plants which produced it, being able to fertilize other plants.

Darwin has furnished many instances of self-sterility. In addition he reports some experiments which led him to believe that even when a plant was normally self-fertile that pollen from unrelated plants of the same species was prepotent over the plant's own pollen. In discussing means which insure flowers being fertilized with pollen from distinct plants, he says: "We now come to a far more general and therefore more important means by which the mutual fertilization of distinct plants is effected, namely, the fertilizing power of pollen from another variety or individual being greater than that of a plant's own pollen. The simplest and best known case of prepotent action in pollen, though it does not bear directly on our present subject, is that of a plant's own pollen over that from a distinct species. If pollen from a distinct species be placed on the stigma of a castrated flower, and then after the interval of several hours, pollen from the same species be placed on the stigma, the effects of the former are wholly obliterated, excepting in some rare cases. If two varieties are treated in the same manner, the result is analogous, though of directly opposite nature; for pollen from any other variety is often or generally prepotent over that from the same flower" ("Cross- and Self-Fertilization," pp. 391-392).

These statements were based on observations and experiments

with various cultivated plants. Different types of crucifers—kohl-rabi, borccoli, Brussels sprouts, cabbage—were grown near each other and the seed resulting from pollination at will, when grown, showed a large amount of intercrossing. The observation was also made with different varieties of the radish, *Raphanus sativus*. These plants are all partially self-sterile so that cross-fertilization is expected in somewhat greater degree than would result from random pollination. Mixing was also shown by plants which are generally self-fertile such as tulip, hyacinth, anemone, ranunculus, strawberry, orange, rhododendron, rub-barb. The fact that vicinism occurs when varieties of these plants are grown together is established by such observations but this does not prove that one type of pollen is prepotent over the other. Somewhat more significant results were obtained from two other species. *Mimulus luteus* was found to be highly fertile when insects were excluded. Uncastrated flowers of a constant whitish variety were artificially pollinated by a yellowish variety and of the 28 resulting plants all had yellowish flowers so that the “pollen of the yellow variety completely overwhelmed that from the mother plant.” A crimson variety of *Iberis umbellata*, which was self-fertile, was crossed with a pink variety, the pollen being applied to uncastrated flowers as before upon the stigmas of which he saw abundant pollen presumably from the same flowers. Out of 30 plants raised 24 showed themselves to be crossed by the altered color of their flowers.

Obviously experiments such as these are not sufficient to establish the prepotency of foreign pollen in self-fertile plants. A number of conclusions might be drawn from such results. The cross-fertilized seeds may have germinated better and the plants grown from them survived in greater numbers. The types may not have been as constant for their flower color as Darwin supposed or the ovules may not have been receptive at the time the plant's own pollen was available but were when the foreign pollen was applied. Taken as they stand the results do indicate a prepotency of pollen from dissimilar plants and it would be desirable to investigate this effect with these species using mixed pollen in reciprocal applications as employed with maize and the tomato.

Darwin knew of many cases of total self-sterility and was so convinced of the necessity for cross-fertilization that he was easily persuaded from these observations that a prepotency of pollen from unrelated plants did exist since he supposed this enabled a plant to choose between its own and unrelated pollen when both were brought at the same time to the stigmas by insects or other agencies. So plausible have been the arguments in favor of such an assumption that the prepotency of germ cells from individuals of somewhat different constitution, even where complete self-fertility exists, has been accepted as an established fact and incorporated in textbooks on biology.

Similarly inconclusive experiments have been performed with animals. Marshall (1910) artificially impregnated a pure bred dog with a mixture of equal quantities of seminal fluids from the same breed and from a mongrel of unknown ancestry. Of the four young which resulted one died early, and three resembled somewhat the mongrel sire. Marshall cites another instance in which a dog copulated with a member of the same breed and two days later with a sire of different type. Out of three puppies one was pure bred and two half-breeds. These cases, according to this writer, indicated a selective action favoring dissimilar rather than related spermatozoa. King (1918) mentions some preliminary experiments with albino and wild gray rats in which advantage was given to the former, yet the results tended to show a prepotency of the latter, so that there was apparently a selective action favoring the out-cross. The details of these experiments are not given.

In attempting to determine whether or not a selective action exists small numbers can never be more than suggestive and unless the mixture is applied at the same time to both types furnishing the sperm cells there is no way of estimating the relative proportions of the two kinds of fertilizing elements present in the mixture which are capable of functioning. Furthermore a constant excess of cross-fertilized individuals over the others may be due to the greater viability of the hybrids and hence there will be a selective elimination of zygotes but not necessarily selective fertilization. Hyde (1914) compared the matings of different types of *Drosophila* within the strains and in reciprocal

crosses. The dissimilar unions gave greatly increased numbers in both reciprocal combinations. However, the type of mating did not influence the number of eggs laid and there is no proof that cross-fertilization occurred more readily than self-fertilization. The results show that the cross-fertilized eggs hatched better and the offspring survived in greater numbers, a result which is easily understood since there were lethal factors involved in the material worked with.

The only evidence from the animal side of a definite selective action comparable to the many instances of self-sterility in plants is the well-known case of self-impotency in *Ciona intestinalis* (Castle, 1896). Morgan (1905, '07, '10) has experimented with this organism and has found that the self-sterility is not always complete. Material gathered on the Pacific coast showed somewhat greater receptiveness to the individual's own sperm than eggs of the same species at Woods Hole which were almost entirely unresponsive to sperm from the same individual which produced the eggs. In another ascidian, *Cynthia partida*, he found that self-fertilization takes place frequently but the sperm of unrelated individuals is more effective. A third species, *Molgula manhattensis* is self-fertilized as readily as cross-fertilized. From this it seems that incompatibility of uniting gametes as a means of insuring cross-fertilization exists in various grades of effectiveness. Even in extreme cases the degree of self-sterility may be modified by internal and external conditions. In *Nicotiana* East and Park find that self-fertilization sometimes takes place towards the end of the growing period when the vigor of the plants is reduced.

DISCUSSION.

As far as the writer knows the results obtained from maize stand alone among plants in showing a selective action unfavorable to fertilization by sperm from individuals of different hereditary constitution. The handicap placed upon the foreign pollen is proportional to the germinal unlikeness. If the unequal effect is due to a slower growth of the pollen tube through the tissues of style the selective action may be restricted to plants,¹

¹ E. C. Miller (Jour. Agric. Research, Vol. 18, pp. 255-266, Dec. 1919), has recently made a detailed study of fertilization in maize and finds that from many

and would also not be surprising to find that the phenomenon is greater in maize than in any other species for the reason that in this plant the pollen tubes have a larger distance to traverse to accomplish fertilization than in any other form known to the writer.

The stigmatic hairs of maize are scattered along a filamentous style which continues to grow until fertilization takes place. The structure withers and dries shortly after pollination takes place. Pollen may adhere at any point along the filament. The total length of style through which the pollen tube grows is normally from 10 to 20 centimeters but in extreme cases may be as great as 50 or more. It will be worth while to see whether or not the selective effect is more pronounced when the styles are long than when short. Such an experiment can be easily carried out and would give some indications as to whether the handicap is placed during pollen tube growth or after the sperm nuclei are brought to the egg.

The lessened ability of moderately different types to fertilize is in line with the impossibility of effecting unions between widely separated forms. In such cases the prevention of fertilization is sometimes due to mechanical difficulties in the way of bringing sperm cells to the eggs but even when this is accomplished there still exists a firm barrier which prevents the passage through the egg membrane. The differential effect demonstrated in maize may be simply a reduced manifestation of this phenomenon.

It is possible that the experiments on anaphyllaxis may throw some light on this problem. It is known that foreign proteins when injected in animal tissues may have a toxic effect and excite an extreme irritability so that in repeated doses they may cause markedly injurious results. By this means it is possible to distinguish between proteins of very slight differences in composition. Since the differences in protoplasmic substances between the types in which a selective action is shown seem to be small there may be some relation between the two phenomena. However, the male gametophyte growing upon the stigmas is, in pollen tubes which start to grow down the style only one tube in every case in nearly 100 observations was seen to reach the ovary cavity. This indicates that the differential fertilizing power is determined by the rate of pollen tube growth and not after the sperm nuclei have been brought to the egg.

a way, merely a parasitic organism. It would be difficult to find evidence from true parasites that they are restrained by their hosts in proportion as they are genetically dissimilar although in most cases the differences are so great that probably there is no basis for comparison.

The only evidence which has any direct bearing on the problem comes from grafting experiments. It is well known with plants that the affinity of stock and cion is directly proportional to their phylogenetic similarity. In animals the same rule holds, and very fine distinctions are manifested. Morgan (1910) cites the results of Schoene in which the skin of the mouse is readily grafted back upon the same individual or member of the same litter but not upon unrelated mice. Such results as these are quite similar to the greater receptiveness of plants to their own pollen.

For some time there has been current in biological literature the hypothesis that heterogeneity in the structure of organisms favors growth and reproduction and conversely that homogeneity is unfavorable. This is a heritage from Darwinism and has appeared again and again in theories of rejuvenation, vigor derived from crossing, and selective fertilization. It has been stated in many different ways but in general terms it amounts to the supposition that similarity in protoplasmic structure brought about by a line of similar ancestors is not conducive to physiological efficiency and that the differences brought about by the union of unlike elements and the consequent lack of balance stimulates growth. The only basis for this hypothesis is found in the necessity, in most cases, for the union of two differentiated sex cells to start the development in the egg. The attempt to argue by analogy that the union of dissimilar protoplasms is, in itself, an immediate physiological benefit is not supported by the facts and is founded upon fallacious reasoning.

Rejuvenation in vegetatively reproduced organisms by sexual union is no longer looked upon as beneficial in destroying similarity in structure. That the process of forming gametes and their reunion may bring about a reorganization of the protoplasmic substances and an elimination of waste products so as to result in greater growth seems quite plausible. But the

significance of such a process is not to be looked for in the bringing together of differentiated germplasms.

The advantage derived from cross-fertilization is now understood as a phenomenon of inheritance and the older hypothesis of the stimulation of heterozygosis is no longer needed. According to present theory homozygous factor combinations are more efficient than heterozygous combinations of the same factors. In the lowest organisms which are illustrative of a primitive sexuality there is direct proof that the union of different individuals does not result in an increased developmental efficiency. Jennings (1913) finds that in *Paramecium*, in the generations immediately following conjugation, there is a slowing down in the rate of division. The advantage derived from the pairing of individuals is the greater elasticity in adaptiveness resulting from the mixing of different germ plasms giving to some of the descendants a greater chance for survival. Pearl (1907) has found that in this same organism there is a tendency for like forms to conjugate due to mechanical hindrances to the pairing of individuals dissimilar in form. This has been substantiated by Jennings (1911). Also in gastropods Crozier (1918) has demonstrated that assortative mating takes place between individuals of the same size and this, he considers, results in a greater number of offspring than there would be if random pairing was the rule. It has been proven by Pearson and Lee (1903) that assortative mating occurs in man. This conclusion is reached after extensive investigation in the inheritance of physical characters in which they have found that there are positive correlations between husband and wife with respect to stature, span of arms, and length of forearm. Moreover they have shown that homogamy is a factor favorable to fertility. Parents with like characters are more productive of offspring. This is an important observation and supports the main thesis of this paper.

The occurrence of homogamy in such widely diverse forms of life as the higher plants, protozoa, mollusca and man cannot fail to have significance. The importance of discriminate isolation has long been recognized since it supplies a necessary factor in divergent evolution. The existence of such an assortative action can now be looked for in all forms of life since evidence has been

produced from the corners of the phylogenetical triangle. The evolutionary significance of this phenomenon has been ably reviewed by Peal in the above citation so that it is not useful to go into that phase of the problem in detail here. It is interesting to note that the results from maize fulfill his expectation as in conclusion he says, "the fact that we find such a high degree of homogamy in a protozoan form like *Paramecium* strongly suggests the possibility that in higher organisms there may be assortative mating of the gametes in the process of fertilization. Should such a homogamy occur it would probably be of far greater importance than any assorted mating of the somas." While the selective agency in plants does not differentiate between gametes produced by one type in the end the result is the same. Individuals with like characters tend to be brought together and virtually to be set apart from the general population.

Biological investigations unite to show that the importance of sex is to make organisms more plastic in adaptability. The advantages have been so great that sexual reproduction is now established as the dominant method for the renewal of organism in both kingdoms at the expense of economy and speed of multiplication. It is not strange then that accessory devices have been developed to insure the fulfillment of the function for which so much has been expended. Self-sterility or self-impotency is one of the means developed to serve this purpose.

The reverse phenomenon, that of self-prepotency, so far is shown among plants only by maize. It would be surprising to find it limited to this one species. Is it not more likely to be a general manifestation? As a fundamental principle it may apply even to those organisms which show self-sterility, this latter being a special adaptation entirely overcoming the handicap placed upon unfamiliar gametes in order to make certain the advantages which exogamy holds out. One cannot insist upon such an assumption with evidence from only one or possibly two species. But the evidence, limited though it may be for the present, is one more indication that homogeneity, similarity, likeness, familiarity, or however it may be described, in protoplasmic structure is consistent with and favorable to the highest physiological efficiency.

In maintaining two opposing tendencies Nature is not necessarily working at cross-purposes. Biparental inheritance with the inclination towards exogamy serves to bring about plasticity. The preferential mating of similar kind operates to make this mixing discriminative. Probably it is not yet time to reconcile completely these two contradictory forces. It may at least be held that assortative mating which favors the pairing of like with like has some importance in evolution since it is an agency in orthogenetic changes. Perceptive reproduction of this kind tends to hold organisms in certain paths once a break from the common type has been made. Having been demonstrated in three of the farthest separated branches of the organic world—angiosperms, protozoa, and man—homogamy may take a somewhat more authoritative part in evolutionary theory.

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